

CLAIMS

1. A bioaffinity assay for quantitative determination in a sample of free PAPP-A,
5 defined as the pregnancy associated plasma protein A (PAPP-A) that is not
complexed to the proform of major basic protein (proMBP), wherein
free PAPP-A is determined either
 - i) as a calculated difference between measured total PAPP-A and measured PAPP-
A complexed to proMBP, or
 - 10 ii) by a direct bioaffinity assay measuring only free PAPP-A.
2. The assay according to claim 1 wherein free PAPP-A is determined according to
alternative i) and two assays are performed, in which one aliquot of the sample is
exposed to a binder which binds total PAPP-A and the binder is detected, and
15 another aliquot of said sample is exposed to a binder which binds only PAPP-A
complexed to proMBP and the binder is detected, and the amount of free PAPP-A is
calculated as a difference between determined total PAPP-A and PAPP-A
complexed to proMBP.
- 20 3. The assay according to claim 2, wherein the assays are non-competitive sandwich
assays.
4. The assay according to claim 3, wherein the binders are capture binders.
- 25 5. The assay according to claim 3, wherein the binders are labelled binders .
6. The assay according to claim 1 wherein free PAPP-A is determined according to
alternative i) as one single dual analyte assay where the sample is exposed to a
capture binderr, which binds total PAPP-A, and to two detecting binders labelled
30 with different labels, so that the first detecting binder labelled with the first label is
directed to an epitope present in any PAPP-A molecule, where the signal of the first
label is used to give total PAPP-A, and the second detecting binder labelled with the

second label is directed to an epitope in the proMBP subunit of the molecule, where the signal of the second label is used to give PAPP-A complexed to proMBP.

5 7. The assay according to claim 1 wherein free PAPP-A is determined according to alternative ii) by exposing the sample to a binder which binds the free PAPP-A but not the PAPP-A complexed to proMBP, and the free PAPP-A bound by said binder is detected.

10 8. The assay according to claim 1 wherein free PAPP-A is determined according to alternative ii) by making PAPP-A complexed to proMBP non-capable of participating in the bioaffinity reaction in which the sample is exposed to a binder binding total PAPP-A.

15 9. The assay according to claim 8 wherein PAPP-A complexed to proMBP is blocked or pre-adsorbed.

10. The immunoassay according to any of the claims 2 to 9 wherein the binder is an antibody, an antibody fragment or an aptamer.

20 11. A method for diagnosing an acute coronary syndrome in a person by using as marker either free PAPP-A as such or a ratio
- free PAPP-A/total PAPP-A,
- free PAPP-A/PAPP-A complexed to proMBP, or
- PAPP-A complexed to proMBP/total PAPP-A.

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12. The method according to claim 11 wherein free PAPP-A is determined according to any of the claims 1-10.

30 13. The method according to claim 11 or 12 wherein total PAPP-A and PAPP-A complexed to proMBP are determined by two different assays according to claims 2 to 5 or in one single assay according to claim 6.

14. The method according to claim 11 or 12 wherein free PAPP-A is determined according to any of the claims 7-10.

15. A binder which binds free PAPP-A but not PAPP-A complexed to proMBP.

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16. The binder according to claim 15 which is an antibody, an antibody fragment or an aptamer.